Supplemental Data for Zandvakili et al.

Sequences used in the GD-lacZ and *RhoAAA-lacZ* reporter assays. Sequences in FASTA format for all the constructs generated and tested in the transgenic reporter assays. Note, the *RhoAAA-lacZ* transgenic reporters contain three concatemers of each listed *RhoA* sequence.

>DCRE_WT

GGAATTTATGTCCGACAATATTTGGGAAATTAAATCATTCCCGCGGACAGTTTTATAGTGCGGGCGTGGC

>DCRE_AF

GGAATTTATGTCCGACAATATTTGGGTGATTGACATTTTTATTATGCGGGCGTGGC

>DCRE_AR

GGAATTTATGTCCGACAATATTTGGGTAATAAAAATGTCAATCATGCGGGCGTGGC

>DCRE_AR_HoxM

GGAATTTATGTCCGACAATATTTGGGTAAGCAAAATGTCAATCATGCGGGCGTGGC

>DCRE_HF

GGAATTTATGTCCGACAATATTTGGGCTATAAAACTGTCCGCGGGAATGATTTAATTTTGCGGGCGTGGC

>DCRE+5

GGAATTTATGTCCGACAATATTTGGTGACCGAAATTAAATCATTCCCGCGGACAGTTTTATAGTGCGGGCGTGGC

>DCRE+10

GGAATTTATGTCCGACAATATTTGGCCGGTACGAAGAAATTAAATCATTCCCGCGGACAGTTTTATAGTGCGGGCG
TGGC

>DCRE+15

GGAATTTATGTCCGACAATATTTGGATGAGAAGCCGTGCCGAAATTAAATCATTCCCGCGGACAGTTTTATAGTGC
GGGCGTGGC

>DCRE+20

GGAATTTATGTCCGACAATATTTGGTGCCTATGAGAAGCCGTGCCGAAATTAAATCATTCCCGCGGACAGTTTTAT
AGTGCGGGCGTGGC

>DCRE_SlpM

GGAATTTATGTCCGACAATCGTTGGGAAATTAAATCATTCCCGCGGACAGTTTTATAGTGCGGGCGTGGC

>DCRE_Bap

GGAATTTATGTCCTGTTTATAAACAGAAATTAAATCATTCCCGCGGACAGTTTTATAGTGCGGGCGTGGC

>DCRE_Eve

GGAATTTATGTCCAAACAAACAAACGAAATTAAATCATTCCCGCGGACAGTTTTATAGTGCGGGCGTGGC

>DCRE_EveRC

GGAATTTATGTCTGTTTGTTTGTTTGAAATTAAATCATTCCCGCGGACAGTTTTATAGTGCGGGCGTGGC

>DCRE_BapC

GGAATTTATGTCCACAAATATTTGTGAAATTAAATCATTCCCGCGGACAGTTTTATAGTGCGGGCGTGGC

>RhoA_DF

TCGTTGCAGTTCATAAATTAAATCATTCCCGCGGACAGTTTTATAGTGCATATTCGCTGGT

>RhoA_DR

TCGTTGCAGTTCATCTATAAAACTGTCCGCGGGAATGATTTAATTTTGCATATTCGCTGGT

>RhoA+5

TCGTTGCAGTTCAGGTCATTGATTGACATTTTTATTATGCATATTCGCTGGT

>RhoA+10

TCGTTGCAGTTCAGCTCGGGTCATTGATTGACATTTTTATTATGCATATTCGCTGGT

Supplemental Figures:

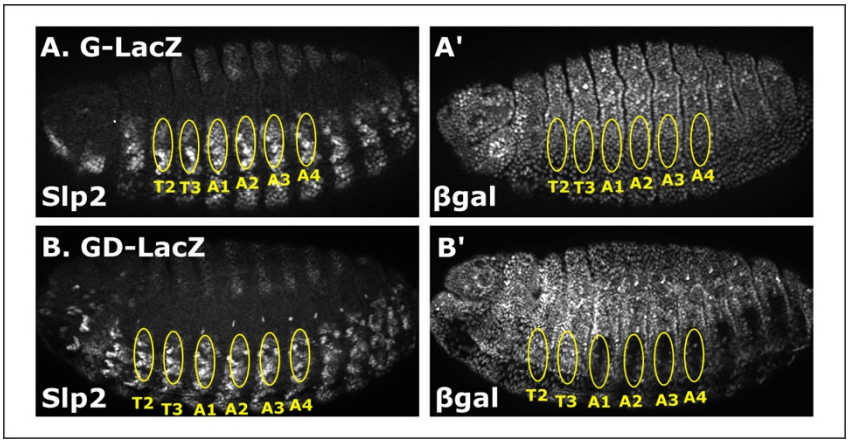

Figure S1. Quantification strategy of β-gal intensity in *3xGBE-DCRE-LacZ* reporter assays.

Lateral views of Stage 15 *Drosophila* embryos stained for Slp2 and β-gal. **(A-B)** Slp2-positive

cells in the lateral ectoderm of the T2-A4 segments were manually encircled to define regions-of-interest (ROIs). **(A'-B')** The ROIs were used to measure β-gal intensity in a separate channel. As explained in the Material and Methods, the mean β-gal intensity measurements were determined by subtracting from background and normalizing to β-gal intensity of the T3 segment.

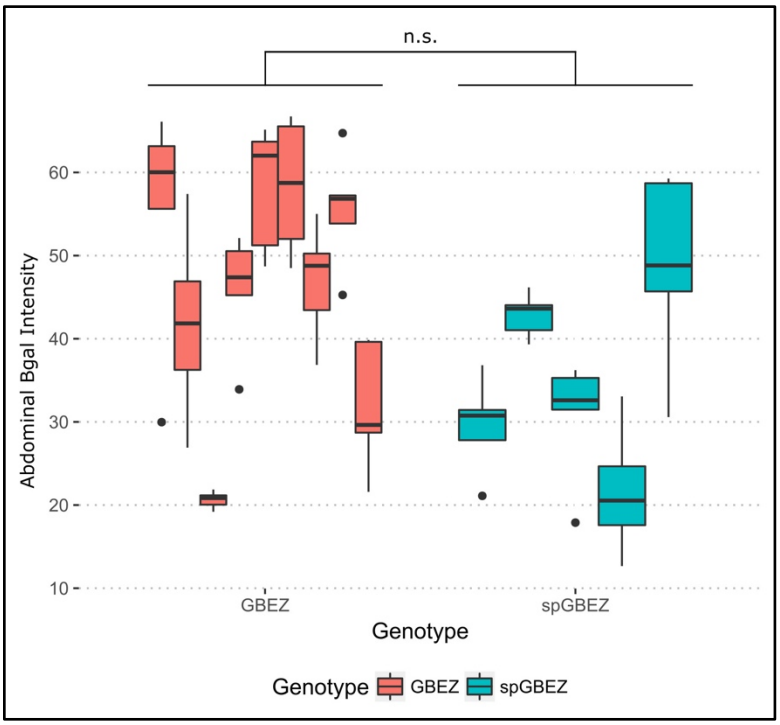

Figure S2. The *sp* sequence is transcriptionally inert. Quantification of β-gal immunostain intensity among abdominal Slp2+ cells in *G-LacZ* vs *spG-LacZ* reporter embryos.

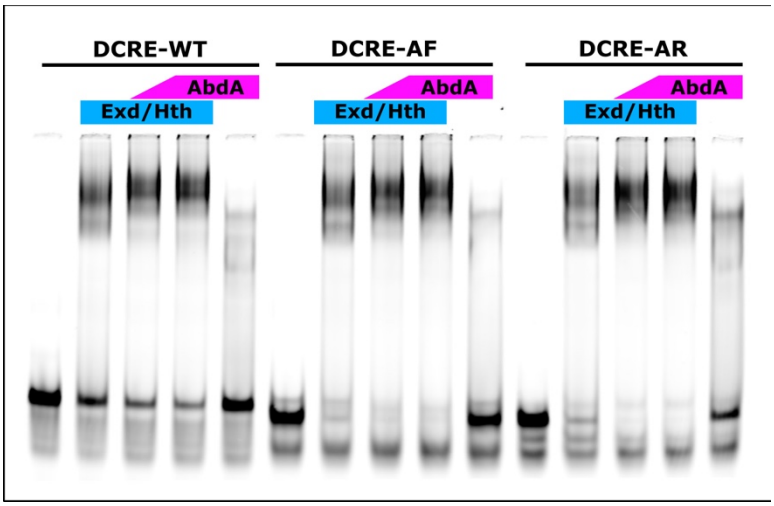

Figure S3. Comparative DNA binding analysis of Exd/Hth/Abd-A on wildtype, AR, and AF variants of *DCRE*. EMSA analysis of Exd/Hth/Abd-A on fluorescent probes of *DCRE* variants (wildtype, AR, and AF, see Figure 3B). Amounts of proteins added were as follows: 59ngs of Exd/Hth; 63ngs and 190ngs of Abd-A.

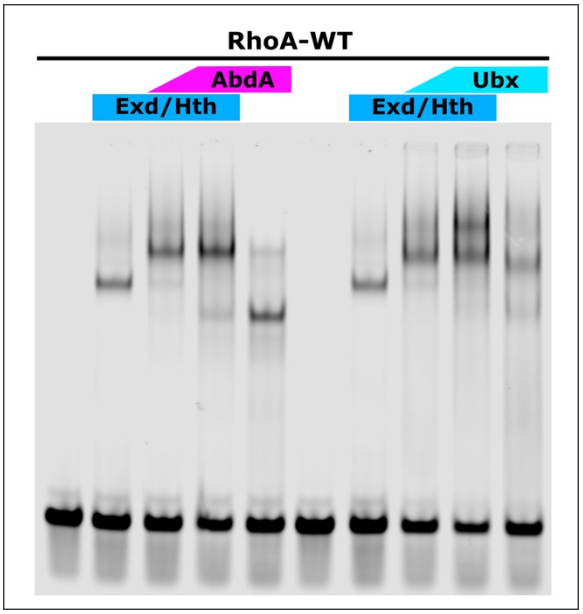

Figure S4. Comparative DNA binding analysis of Exd/Hth/Abd-A and Exd/Hth/Ubx on *RhoA*. EMSA analysis of Exd/Hth/Abd-A versus Exd/Hth/Ubx binding to fluorescent *RhoA* probe.

Amounts of proteins added were as follows: 59ngs of Exd/Hth; 63ngs and 190ngs of Abd-A; 80ngs and 240ngs of Ubx.

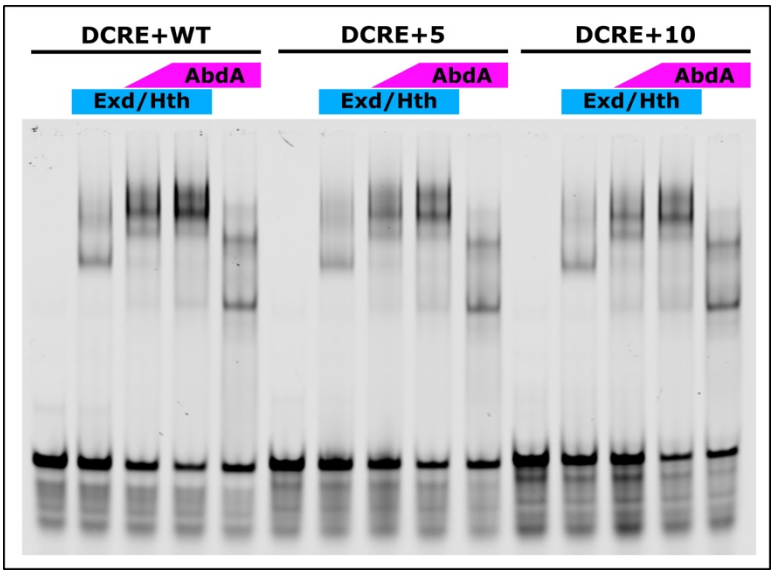

Figure S5. Comparative DNA binding analysis of Exd/Hth/Abd-A on wildtype, +5, and +10 variants of *DCRE*. EMSA analysis of Exd/Hth/Abd-A on fluorescent probes of *DCRE* variants (wildtype, +5, and +10, see Figure 4A). Amounts of proteins added were as follows: 59ngs of Exd/Hth; 63ngs and 190ngs of Abd-A.

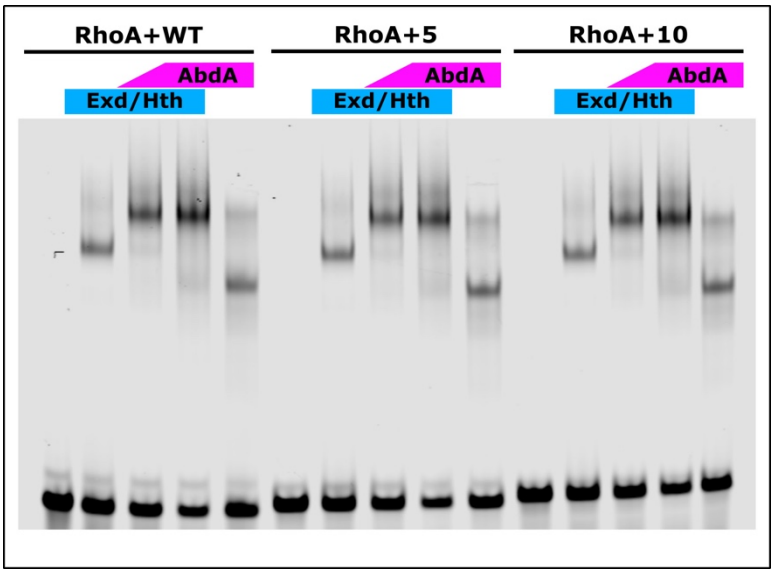

Figure S6. Comparative DNA binding analysis of Exd/Hth/Abd-A on wildtype, +5, and +10 variants of *RhoA*. EMSA analysis of Exd/Hth/Abd-A on fluorescent probes of *RhoA* variants (wildtype, +5, and +10, see Figure 5E). Amounts of proteins added were as follows: 59ngs of Exd/Hth; 63ngs and 190ngs of Abd-A.

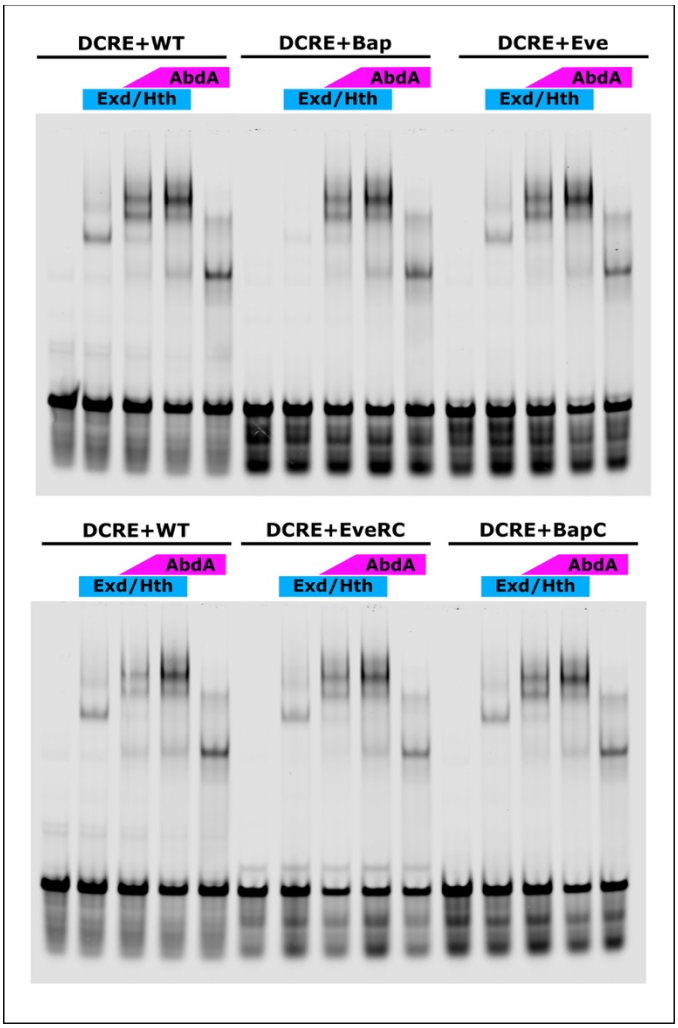

Figure S7. Comparative DNA binding analysis of Exd/Hth/Abd-A on Slp2 binding site variants of *DCRE*. EMSA analysis of Exd/Hth/Abd-A on fluorescent probes of *DCRE* variants (wildtype, Bap, Eve, EveRC, and BapC, see Figure 6A). Amounts of proteins added were as follows: 59ngs of Exd/Hth; 63ngs and 190ngs of Abd-A.